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Association between osteoprotegerin and RANKL single nucleotide polymorphisms and destructive rhinosinusitis in patients with granulomatosis with polyangiitis

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Abstract

Background Chronic invasive rhinosinusitis with facial bone damage is a common cause of functional and social impairment in granulomatosis with polyangiitis (GPA) patients. To the best of our knowledge, there is no clinical or laboratory biomarker to predict bone damage.

Methods This case-control study included 90 patients with GPA and 270 health controls (HCs). Patients were categorized according to the presence of tomographic facial bone erosions. Frequency of RANKL and osteoprotegerin single nucleotide polymorphisms (SNPs), analyzed by real-time polymerase chain reaction, were compared between patients and HCs, and between patients with and without bone damage. Clinical, therapeutic, and laboratory data were analyzed.

Results Facial bone erosion was observed in 55.5% of patients. No difference was found in the frequency of SNPs between patients with GPA and HCs. GPA patients were compared according to the presence or absence of bone damage, and a difference was found in the frequencies of osteoprotegerin G1181C (rs2073618) and RANKL A290G (rs2277438). A multivariate analysis showed that the CC genotype of osteoprotegerin 1181 was independently associated with bone erosion (OR = 3.95, CI95%=1.20–13.00, $P = 0.02$), as were the presence of the G allele in RANKL A290G (OR = 6.13, CI95%=1.95–19.26, $P = 0.002$) and higher disease duration (OR = 1.08, CI95%=1,01-1.15, $P = 0.04$).

Conclusion SNPs in osteoprotegerin G1181C and RANKL A290G may play a role in the development of destructive rhinosinusitis in patients with GPA. Genetic assessment may be useful for identifying high-risk individuals. This observational study might work as a basis for further research to better understand this association and clinical trials using RANKL/osteoprotegerin as therapeutic targets.

Keywords Bone erosion, Granulomatosis with polyangiitis, Osteoprotegerin, Polymorphisms, Rhinosinusitis, Vasculitis, ANCA-associated vasculitis

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Background

Granulomatosis with polyangiitis (GPA) is a systemic autoimmune disease characterized by necrotizing vasculitis and granulomatous inflammation in small and medium-sized vessels [1].

Classically, upper and lower airway involvement and necrotizing pauci-immune glomerulonephritis are the hallmarks of GPA, present in 85% and 70% of cases, respectively [2, 3]. In addition, chronic invasive rhinosinusitis with bone and cartilage damage is common in GPA, usually starting at the nasal septum and spreading to the paranasal sinuses and orbital walls [4, 5]. It leads to significant functional and social impairments [6]. However, knowledge regarding the causes and mechanisms of bone destruction in GPA is limited.

Bone changes at sites of inflammation have been a topic of interest in other rheumatic diseases, particularly in rheumatoid arthritis (RA). The detection of nuclear factor activator receptor *kappa* B (RANK) and its ligand (RANKL) allowed a better understanding of the bone-remodeling process. RANKL is secreted by osteoblasts and osteocytes, and binds to RANK in osteoclasts, thus activating them to initiate the bone resorption process. This interaction is inhibited by osteoprotegerin (OPG) [7, 8]. The immunological role of the RANK-RANKL pathway has been described in RA, in which RANKL is secreted by activated T cells and triggers osteoclasts in the inflamed synovial environment, leading to the typical marginal bone erosion of the disease [9, 10]. Additionally, Takayanagi et al. [11]. found that RANKL mRNA was highly expressed in all tissues from patients with RA but not in those from patients with osteoarthritis. They also demonstrated that cultured rheumatoid synovial fibroblasts efficiently induced osteoclastogenesis via upregulation of RANKL, also decreasing OPG expression, and that osteoclastogenesis was inhibited by OPG in a dose-dependent manner.

Single nucleotide polymorphisms (SNPs), which are variations in the genetic sequence of a single nucleotide [12] in RANK, RANKL, or OPG, can affect their encoded proteins. Since their discovery over the past 20 years, several SNPs have been implicated in genetic susceptibility to osteoporosis and osteoporotic fractures [13–16]. Moreover, a meta-analysis involving three French cohorts of patients with RA identified one SNP on RANK, one haplotype on RANKL associated with the presence of an anti-citrullinated peptide antibody, and one SNP on OPG associated with erosion [10]. In this context, a hypothesis arises that RANKL and OPG SNPs may have a role in the pathogenesis of facial bone changes in GPA, which provides a possible genetic explanation for the susceptibility of selected individuals to this specific involvement of the disease.

Therefore, this study sought to analyze the association between OPG and RANKL polymorphisms and bone damage in the upper respiratory tract of patients with GPA. Furthermore, clinical data, laboratory parameters, and therapeutic features that could be related to bone injury were evaluated.

Methods

Study design

This case-control study included patients with GPA in regular follow-up at the vasculitis outpatient clinic from the Rheumatology or Pneumology Divisions at our tertiary referral hospital between July 2019 and July 2022. The study was approved by the local research and ethics committee (CAAE 50007521.1.0000.0068) and all participants gave written informed consent.

Patients

GPA was defined according to the American College of Rheumatology (ACR) classification criteria (1990) [17] or Chapel Hill Conference Definition Criteria (2012) [18]. In addition, all patients were retrospectively classified according the to the ACR/European Alliance of Associations for Rheumatology (EULAR) 2022 criteria for GPA [19]. Both childhood-onset and adult-onset GPA subjects were included.

Exclusion criteria

Patients with chronic rhinosinusitis or facial bone damage that could be attributed to an alternative diagnosis such as lymphoma, surgery, cocaine abuse, or fungal infection were excluded from the study.

Control individuals

The healthy controls (HC) were enrolled from a previous cohort study [20, 21] with the required data of interest and genotyping in a 1 patient:3 controls ratio, matched by sex and ethnicity.

Clinical data and laboratory parameters

Patients' medical history, including habits, disease duration, disease extent, anti-neutrophil cytoplasmic antibody (ANCA) status, and therapeutic approaches, were acquired during one of their regular outpatient visits through interviews and medical record revision. Ethnicity was defined based on the self-reported ethnicity of second-generation ancestors, an approach previously used for Brazilian populations [22, 23]. ANCA was tested by indirect immunofluorescence, while anti-PR3 and anti-MPO antibodies were analyzed by enzyme-linked immunosorbent assay (ELISA). ANCA and anti-proteinase 3 (PR3) were considered positive if positive at any time along the disease course. For the analysis of previous

therapeutic approaches, cumulative doses of cyclophosphamide and rituximab were considered from diagnosis to the date of the tomography, while the cumulative dose of glucocorticoid was evaluated in the 12-month period before the tomography that was considered for the analysis.

Peripheral blood samples were collected from each participant to DNA extraction and SNP genotyping.

Facial bone damage evaluation

According to presence of bone damage (nasal septum, paranasal sinuses, orbital wall, and temporal bone) defined by computed tomography (CT), patients with GPA were categorized into two groups: those with bone damage (GPA with erosion), and those with no bone damage (GPA without erosion). CT scans were analyzed by two experienced radiologists blinded to the genotyping results. Patients with more than one CT exam had the most recent one considered for analysis. For patients who underwent sinus surgery, which could be a confounding factor for vasculitis-related bone damage, a CT performed before the procedure was chosen for analysis.

SNP genotyping

Genomic DNA was isolated from peripheral blood leukocytes using a Qiagen DNA extraction kit (QIAamp DNA Blood Mini Kit, 51104) and stored at −20 °C prior to analysis. The SNPs studied were chosen according to their possible associations with bone mass, fracture risk, and bone erosion in RA, based on previous data in the literature [24–27]. The selected SNPs were 1181 G > C (rs2073618), located in exon 1 of OPG; 245 T > G (rs3134069), 163 C > T (rs3102735), and 209 G > A (rs3134070), located in the promoter of OPG (chromosomal location 8q24); and RANKL 290 A > G rs2277438, located in the 5′ untranslated region (UTR) of RANKL (13q14).

Genotyping was performed using custom Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA) with allele discrimination and StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Products were amplified in a 10-μL reaction, and the cycling conditions consisted of 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 01 min. All the analysis were performed by the same well-trained technician.

Bone mineral density evaluation

For evaluation of a possible relationship between the presence of facial bone erosions and systemic bone mineral density (BMD), bone mineral density data from the lumbar spine and femur of patients who had already undergone at least one bone densitometry (DXA) during outpatient follow-up were analysed. For patients with more than one DXA available, the one closest to the bone tomography analysed in the study was considered.

Statistical analysis

The Shapiro-Wilk test was used to verify the normal distribution of the variables. Data are expressed as mean ± standard deviation (SD), median (25th −75th IQR), and frequency (%). Comparisons between patients and control groups, and between patients with and without tomographic facial bone damage were performed using Student's t, Mann-Whitney U, chi-square, and Fisher's exact tests. Multiple logistic regression models were used to analyze the variables independently associated with bone erosion. Hardy-Weinberg equilibrium was assessed in patients and controls by comparing the observed SNP distribution with the expected distribution using an exact test of Hardy–Weinberg equilibrium as described in Wigginton et al. [28]. and implemented in the R package SNPassoc [29]. Derived from mathematical equations to support the theory of Mendelian inheritance, the Hardy-Weinberg test proves that in a large population of individuals subjected to random mating, the proportions of alleles and genotypes at a locus remain unchanged, unless specific disturbing influences are introduced. This has been widely recommended as a crucial step in genetic association studies. The analysis of genetic association was based on generalized linear models using the SNPassoc package. Softwares used for statistical analysis were SPSS for Windows (version 20.0; SPSS, Chicago, IL, USA) and R software [30]. Statistical significance was defined as $P < 0.05$.

Results

This study included 90 patients with GPA and 270 sex- and ethnicity-matched HCs (Table 1). The mean age of patients with GPA at disease onset was 43.5 (36–53) years, and average disease duration was 7.0 (1.7–13.2) years. ANCA was positive in 86% of the patients and anti-proteinase 3 (PR3) was positive in 63.4% of the individuals tested for it. 27% had a localized GPA form, and 50% had renal involvement.

Facial bone erosion was observed in 50 of 90 (55.5%) patients with GPA, primarily in the paranasal sinuses ($n = 35$), nasal septum ($n = 34$), and orbital wall ($n = 9$).

There were no differences regarding sex, ethnicity, age at GPA onset, or smoking history between the patients with and without bone erosion (Table 2). Patients with bone damage had a higher disease duration than those without bone destruction (9 *versus*. 3 years, $P = 0.04$). However, ANCA positivity, disease extent (localized vs. systemic), renal involvement rate, and therapeutic approach (previous use and cumulative doses of

Table 1 Demographic and clinical data of patients with granulomatosis with polyangiitis (GPA) and individuals in the control group

	GPA (*n* = 90)	Heath control (*n* = 270)
Female sex	59 (65.5)	177 (65.5)
White ethnicity	70 (77.8)	210 (77.8)
Age (years)	52.0 (43.7–62.0)	69.0 (68.0–70.0)
Age at diagnosis (years)	43.5 (36–53)	-
Disease duration (years)	7.0 (1.7–13.2)	-
Positive ANCA ANCA subtype – C-ANCA	78 (86.7) 67 (85.9)	-
Localized GPA	25 (27.7)	
Bone erosion	50 (55.5)	
Renal involvement	45 (50.0)	

Data are expressed as the median (25th – 75th) or frequency (%)

ANCA anti-neutrophil cytoplasmic antibody

Table 2 Comparison of GPA patients classified according to the presence or absence of bone erosions documented by computed tomography

	GPA with erosion (*n* = 50)	GPA without erosion (*n* = 40)	*P* value
Female	31 (62.0)	28 (70.0)	0.43
White ethnicity	39 (78.0)	31 (77.5)	0.95
Smoking history	12 (0.2)	13 (0.3)	0.37
Age at diagnosis (years)	43.0 (37.5–52.0)	45.5 (36.0-58.5)	0.52
Disease duration (years)	9.0 (3.7–15.0)	3.0 (1.0-11.7)	0.04
Positive ANCA	43 (86.0)	35 (87.5)	1.00
Localized GPA	14 (28.0)	11 (27.5)	0.96
Renal involvement	25 (50.0)	20 (50.0)	1.00
Creatinine (mg/dL)	0.83 (0.72–1.21)	0.87 (0.71–1.09)	0.61
CRP (mg/L)	3.45 (1.8–9.7)	3.4 (1.1–11.9)	0.71
Cumulative GC dose (g)	1.67 (0-5.88)	1.50 (0-4.29)	0.81
CYC use	33 (66.6)	27 (67.5)	0.88
Cumulative CYC dose (g)	6.7 (0-15.6)	7.5 (0-34.8)	0.48
RTX use	19 (38.0)	11 (27.5)	0.29
Cumulative RTX dose (g)	0 (0–5)	0 (0–2)	0.18

Data are expressed as the median (25th – 75th) or frequency (%)

GPA granulomatosis with polyangiitis, *ANCA* anti-neutrophil cytoplasmic antibody, *CRP* C-reactive protein, *GC* glucocorticoid, *CYC* cyclophosphamide, *RTX* rituximab

cyclophosphamide and rituximab) were similar between both groups.

No difference was found in the frequency of the five SNPs analyzed between the 90 patients with GPA and the 270 HCs (supplementary table). Patients with GPA did not deviate from the Hardy-Weinberg equilibrium for any of the genes analyzed. However, in the OPG 209 (rs3134070) analysis, the control group population deviated from the Hardy-Weinberg equilibrium.

In the comparison between GPA patients with and without facial bone destruction, it was found a difference in the frequency of two SNPs: OPG 1181 G > C (rs2073618) and RANKL 290 A > G (rs2277438) (Table 3). For OPG 1181, 40% of the patients with erosion had the CC genotype vs. 17.5% of patients without facial erosion (OR = 4.29, CI95% 1.24–14.83, $P = 0.04$). In the recessive model, which compared the CC genotype with the GG and GC genotypes grouped together, a 3.14 times-increase in the risk of erosion was observed in the CC group (CI 95% 1.16–8.48, $P = 0.01$). Regarding RANKL 290 A > G, patients with erosion had a higher frequency of the G allele than those without erosion (60.0% vs. 17.5%, $P = 0.0005$). The presence of this allele, either in homozygous (GG) or heterozygous (AG), was associated with a 4.71 times-increase in the risk of erosions (CI 95%=1.76–12.64, $P = 0.001$). Only 5 patients were homozygous (GG) for RANKL 290 A > G, and all of them had bone erosions. The frequencies of the other three SNPs tested (OPG 209 C > T, OPG 163 C > T, and OPG 245 A > C) were similar between the patients with and without bone damage.

The concomitance of the two SNPs associated with a higher frequency of bone erosion (CC for OPG rs2073618 and, AG or GG for RANKL rs2277438) was observed in 11 patients with GPA, and all had bone erosions. In contrast, 42 of 90 patients with GPA had neither of these two SNPs, and the frequency of erosion in this subgroup was 38.1%.

The demographic and clinical characteristics of patients with GPA and the CC genotype for OPG 1181 (rs2073618) were compared to those of patients with the GG and GC genotypes. In addition to the higher frequency of facial bone erosion in the CC group (74.1% vs. 47.6%, $P = 0.02$), less white individuals were observed in the CC group (63% vs. 84.1%, $P = 0.03$) (Supplementary Table 2). The same analysis was performed for RANKL 290 (rs2277438), comparing individuals according to the presence of allele G (genotypes AG or GG) and those without it (genotype AA). The only difference observed was a higher frequency of bone damage in patients carrying the G allele (78.1% vs. 43.1%, $P = 0.001$) (Supplementary Table 3).

Finally, a binary logistic regression was performed in a model including the results of the genetic testing in the univariate analysis adjusted for variables considered clinically important for the development of bone erosions: duration of GPA and cumulative dose

Table 3 Comparison of the frequency of the analyzed SNPs between patients with granulomatosis with polyangiitis with and without bone damage

SNPs	With erosions (*n* = 50)	Without erosions (*n* = 40)	OR (CI 95%)	*P*-value
OPG 209 C > T (rs3134070)				
Codominant				
- CC	43 (86.0)	38 (95.0)	1.00	0.36
- CT	6 (12)	2 (5.0)	2.65 (0.50-13.93)	
- TT	1 (2.0)	0 (0)		
Allele C	92	78		0.19
Allele T	8	2		
OPG 163 C > T (rs3102735)				
Codominant				
- TT	39 (78.0)	28 (70.0)	1.00	0.54
- CT	9 (18.0)	11 (27.5)	0.59 (0.21–1.61)	
- CC	2 (4.0)	1 (2.5)	1.44 (0.12–16.62)	
Allele C	87	67		0.54
Allele T	13	13		
OPG 1181 G > C (rs2073618)				
Codominant				
- GG	8 (16.0)	12 (30.0)	1.00	0.04
- CG	22 (44.0)	21 (52.5)	1.57 (0.54–4.61)	
- CC	20 (40.0	7 (17.5)	4.29 (1.24–14.83)	
Allele G	38	45		0.01
Allele C	62	35		
Dominant				
GG	8 (16.0)	12 (30.0)	1.00	0.11
CG/CC	42 (84.)	28 (70.0)	2.25 (0.82–6.20)	
Recessive				
GG/CG	30 (60.)	33 (82.5)	1.00	0.002
CC	20 (40.0	7 (17.5)	3.14 (1.16–8.48)	
OPG 245 A > C (rs3134069)				
Codominant				
- AA	43 (86.0)	38 (95.0)	1.00	0.36
- AC	6 (12.0)	2 (5.0)	2.65 (0.50-13.93)	
- CC	1 (2.0)	0 (0)		
Allele A	92	78		0.19
Allele C	8	2		
Dominant				
AA	43 (86.0)	38 (95.0)	1.00	0.14
AC/CC	7 (14.0)	2 (5.0)	3.09 (0.61–15.8)	
RANKL 290 A > G (rs2277438)				
Codominant				
- AA	25 (50.0)	33 (82.5)	1.00	0.002
- AG	20 (40.0)	7 (17.5)	3.77 (1.38–10.31)	
- GG	5 (10.0)	0		
Allele A	70	73		0.0005
Allee G	30	7		
Dominant				
AA	25 (50.0)	33 (82.5)	1.00	0.001
AG-GG	25 (50.0)	7 (17.5)	4.71 (1.76–12.64)	

SNPs single nucleotide polymorphisms, *OPG* osteoprotegerin, *RANKL* nuclear factor activator receptor kappa B, *OR* odds ratio

Table 4 Binary logistic regression of variables possibly associated with the development of bone damage in patients with granulomatosis with polyangiitis (GPA)

	GPA with erosion (*n* = 50)	GPA without erosion (*n* = 40)	OR	CI95%	*P* value
Female	31 (62.0)	28 (70.0)	0.41	0.14–1.16	0.09
Disease duration (years)	9.0 (3.7–15.0)	3.0 (1.0-11.7)	1.08	1.01–1.15	**0.04**
Cumulative CYC dose (g)	6.7 (0-15.6)	7.5 (0-34.8)	0.99	0.98-1.00	0.13
Cumulative RTX dose (g)	0 (0–5)	0 (0–2)	1.09	0.94–1.26	0.26
Genotype CC OPG 1181 (rs2073618)	20 (40.0)	7 (17.5)	3.95	1.20–13.00	**0.02**
Allele G RANKL 290 (rs2277438)	30 (60.0)	7 (17.5)	6.13	1.95–19.26	**0.002**

Data are expressed as the median (25th – 75th) or frequency (%)

CYC cyclophosphamide, *RTX* rituximab, *OPG* osteoprotegerin, *RANKL* nuclear factor activator receptor kappa B, *OR* odds ratio, *CI* confidence interval

Table 5 Sub-analysis of patients with GPA with and without facial bone erosions with disease duration longer than 3 years

	GPA with erosion (*n* = 27)	GPA without erosion (*n* = 19)	*P* value
Disease duration (years)	9.0 (5–15)	12 (4–12)	0.13
Cumulative CYC dose (g)	7 (0-21.7)	22.5 (0–54)	0.11
Cumulative RTX dose (g)	0 (0–6)	0 (0–4)	0.42
Genotype CC OPG 1181 (rs2073618)	12 (44.4)	2 (10.5)	0.01
Allele G RANKL 290 (rs2277438)	12 (44.4)	3 (15.8)	0.04

Data are expressed as the median (25th – 75th) or frequency (%)

CYC cyclophosphamide, *RTX* rituximab, *OPG* osteoprotegerin, *RANKL* nuclear factor activator receptor kappa B, *OR* odds ratio, *CI* confidence interval

of cyclophosphamide and rituximab (Table 4). In this analysis, the CC genotype for OPG 1181 was independently associated with bone erosion in patients with GPA (OR = 3.95, CI95%=1.20–13.00, $P = 0.02$), as well as the presence of the G allele for RANKL 290 (OR = 6.13, CI95%=1.95–19.26, $P = 0.002$) and disease duration (OR = 1.08, CI95%=1,01-1.15, $P = 0.04$). In order to reduce the bias of disease duration, since an individual may develop bone erosions at any time point during the disease course and individuals without erosions had shorter disease duration compared to individuals with erosions, a sub-analysis including only patients with disease course higher than 3 years ($n = 46$) was performed. In this analysis disease duration was similar between patients with and without erosions (9 vs. 12 years, $p = 0.13$) and the 2 SNPs remained independently associated with erosions (genotype CC vs. genotypes CG and GG for OPG C1181C - $P = 0.01$; genotypes AG and GG vs. genotype AA for RANKL A290G - $P = 0.04$) (Table 5).

For the investigation a possible relationship between facial bone erosions and systemic bone mineral density, an evaluation was carried out on patients who had already undergone a DXA examination at some point during follow-up. 50 of 90 of the patients with GPA (55%) had DXA available. Of these, 13 had normal bone mineral density, 19 had osteopenia and 18 had osteoporosis. No difference was found between bone mineral densities (in g/cm2) of the femoral neck, total femur and lumbar spine between patients with GPA with and without facial bone erosions [0.746 vs. 0.637 ($p = 0.17$), 0.906 vs. 0.787 ($p = 0.5$) and 0.855 vs. 0.819 ($p = 0.3$), respectively]. BMD of the femoral neck, total femur and lumbar spine were also similar when comparing patients with the CC genotype of the OPG 1181 gene, associated with a higher frequency of facial bone erosions, with patients with the GC and GG genotypes [femoral neck: 0.797 vs. 0.686 g/cm2 ($p = 0.26$); total femur: 0.916 vs. 0.846 g/cm2 ($p = 0.39$); lumbar spine: 0.858 vs. 0.838 g/cm2 ($p = 0.24$)]. Likewise, there was no difference in bone mineral density at these sites between patients with the AG and GG genotypes of the RANKL 290 gene, associated with a higher frequency of facial bone erosions, and patients with the AA genotype [femoral neck: 0.742 vs. 0.688 g/cm2 ($p = 0.97$); total femur: 0.908 vs. 0.852 g/cm2 ($p = 0.83$); lumbar spine: 0.843 vs. 0.844 g/cm2 ($p = 1.0$)].

Discussion

To the best of our knowledge, this was the first study that analyzed bone-related SNPs in GPA patients. We demonstrated an association between two single-nucleotide

polymorphisms located in the OPG and the RANKL genes and facial bone damage in patients with GPA. It was found no difference between the frequency of the analyzed SNPs comparing GPA-patients with no bone damage and controls without GPA. Among patients with GPA, higher disease duration was also associated with facial erosion, which can be explained by the time required for the development of bone erosion. However, no other clinical, demographic, or laboratory features, including the therapeutic approach, was associated with presence of bone destruction. Regarding the therapeutic approach, the cumulative dose of glucocorticoid was evaluated in the 12-month period before the tomography that was considered for the analysis. The choice of this period was based on the fact that the cumulative dose of glucocorticoid used by each patient since diagnosis is an unreliable data for a retrospective analysis, with a large percentage of missing information, since patients are referred to the tertiary service already using glucocorticoids for a variable period of time, and many patients used them irregularly without medical supervision before starting the regular follow-up.

The presence of the G allele on RANKL 290 (rs2277438), whether heterozygous or homozygous, had the strongest association with bone damage in GPA, with an odds-ratio of 6.13. The rs2277438 is located in intron 1, a potential regulatory region of RANKL, but its effect on the regulation of RANKL expression remains unclear [31]. Interestingly, the presence of the G allele in this gene was previously associated with the risk of developing ankylosing spondylitis, a disease known to cause bone destruction and, more typically, bone formation, in a Chinese population [32]. Several studies have evaluated the role of RANKL SNPs in RA, a classic erosive disease. Yang et al. found that SNP in *RANKL* gene (rs2277438) increased the risk of RA [33]. A previous study by Xu et al. demonstrated that this RANKL polymorphism may not be a susceptibility factor for RA in a Chinese Han population, but may play a significant role in typical bone and joint injuries of the disease [34]. This RANKL SNP (rs2277438) was previously associated with radiographic progression over two years in a Japanese cohort of early RA subjects [35].

Concerning to the SNP on OPG 1181 (rs2073618), in the present study the CC genotype resulted in a 3.95-fold increase in the risk of developing bone erosions in patients with GPA. The rs2073618 is in the first exon of OPG and substitutes asparagine for lysine [36]. This SNP could have a functional effect, and thus, could be implicated in the pathophysiology of bone erosion. In a large meta-analysis of three French cohorts, OPG rs2073618 was significantly associated with erosion in RA [10]. Interestingly, another meta-analysis of eight studies demonstrated that the OPG G1181C polymorphism is associated with lumbar bone mineral density (BMD) in Europeans and Asians and with femoral neck and total hip BMD in Europeans, but with the GG genotype resulting in lower BMD values [37]. Among Indian women, the SNP of OPG rs2073618 was demonstrated to be a predictor of spine BMD, with the genotype CC resulting in lower bone mass [38]. In a large cohort of Arab women, occurrence of CG genotype and the combination of GG + CG genotypes in OPG rs2073618 was associated with a 40% lower risk of developing osteoporosis compared to individuals with the CC genotype. It was also observed that those with the G allele had a 30% lower risk of osteoporosis than individuals with the C allele [39]. Therefore, despite some conflicting results, most of these findings are in line with our results, suggesting that the G allele is probably protective against bone loss.

The exact mechanism by which these SNPs lead to changes in bone metabolism is not yet fully understood. Transgenic OPG null mice develop severe osteoporosis, whereas mice overexpressing OPG have an osteopetrosis phenotype, indicating that OPG blocks osteoclast formation and bone resorption [40]. Similarly, transgenic animal models, including knockout of RANKL, exhibit severe osteopetrosis. These animals have a complete block in osteoclastogenesis, which can be restored after the reintroduction of the RANKL gene into bone marrow progenitor cells [41, 42]. In this study the serum levels of OPG and RANKL were not evaluated, and previous surveys did not correlate OPG and RANKL SNPs with serum levels of OPG and RANKL [16, 43]. RANKL expressed on activated T cells can trigger osteoclast activation, and RANKL/RANK may play a major role in inflammation-induced bone loss and joint destruction in arthritis. It is well established that RANKL system components are present in the rheumatoid synovium and osteoclasts have a major role in rheumatoid bone erosion [44–46]. In this way, local alterations in the RANKL: OPG ratio rather than systemic changes are critical determinants of bone damage [9].

The strengths of the present study are (a) we included a representative sample of patients with a rare disease, (b) the use of the newly published classification criteria for GPA, and (c) the use of easy-to-measure binary variables leading to strong associations among the analyzed disease parameters. A possible limitation of this study is the age disparity between patients and controls. It should be noted, however, that the older age of individuals in the control group is not a limitation for analysis of innate polymorphisms, and other clinical

or epidemiological variables that could be influenced by this aspect were not the focus of this study. On the other hand, this age difference decreases the chance of an individual in the control group developing GPA, which would place them in the other group. Furthermore, the relevant findings of this study were observed comparing patients with GPA with and without facial bone erosions, not involving the control group.

Conclusion

In conclusion, SNPs in osteometabolism-related genes, specifically OPG G1181C (rs2073618) and RANKL A290G (rs2277438), may play a role in the development of destructive rhinosinusitis in patients with GPA. Genetic assessment of subjects with GPA, although not yet available in clinical practice, could be a useful tool to identify individuals at higher risk of bone damage in order to increase surveillance of this aspect of the disease. This observational study might be useful as a basis for further research to better understand this association as well as clinical trials aimed to evaluate RANKL/osteoprotegerin as therapeutic targets.

Abbreviations

ACR	American College of Rheumatology
ANCA	Anti-neutrophil Cytoplasmic Antibody
BMD	Bone Mineral Density
CRP	C-reactive Protein
CT	Computed Tomography
CYC	Cyclophosphamide
DNA	Deoxyribonucleic Acid
EULAR	European Alliance of Associations for Rheumatology
GC	Glucocorticoid
GPA	Granulomatosis with Polyangiitis
HC	Healthy Controls
OPG	Osteoprotegerin
PCR	Polymerase Chain Reaction
PR3	Proteinase 3
RA	Rheumatoid Arthritis
RANK	Nuclear Factor Activator Receptor Kappa B
RANKL	Nuclear Factor Activator Receptor kappa B ligand
RTX	Rituximab
SNP	Single Nucleotide Polymorphism

Supplementary Information

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Supplementary Material 1

Authors' contributions

MADF and RMRP conceived the study idea and wrote the manuscript. MADF and HAMG collected data and cared for study patients. BWH performed the genetic analysis and served as scientific advisor. VFC performed the laboratory tests and served as scientific advisor. CSVB provided study patients and served as scientific advisor. DSD, SKS and RMRP critically reviewed the study proposal and the results and served as scientific advisors. All authors discussed the results and contributed to the final manuscript.

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Data availability

Full study data are readily available upon email request.

Declarations

Ethics approval and consent to participate

The study was approved by the local research and ethics committee (University of Sao Paulo/Plataforma Brasil CAAE 50007521.1.0000.0068 / approval n. 4.916.501) and all participants gave written informed consent.

Competing interests

The authors declare no competing interests.

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